

Effect of The Receptor Activator of Nuclear Factor κ B and RANK Ligand on *In Vitro* Differentiation of Cord Blood CD133⁺ Hematopoietic Stem Cells to Osteoclasts

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Received: 15/Mar/2015, Accepted: 2/Jan/2016

Abstract

Objective: Receptor activator of nuclear factor-kappa B ligand (RANKL) appears to be an osteoclast-activating factor, bearing an important role in the pathogenesis of multiple myeloma. Some studies demonstrated that U-266 myeloma cell line and primary myeloma cells expressed RANK and RANKL. It had been reported that the expression of myeloid and monocytoid markers was increased by co-culturing myeloma cells with hematopoietic stem cells (HSCs). This study also attempted to show the molecular mechanism of RANK and RANKL on differentiation capability of human cord blood HSC to osteoclast, as well as expression of calcitonin receptor (CTR) on cord blood HSC surface.

Materials and Methods: In this experimental study, CD133⁺ hematopoietic stem cells were isolated from umbilical cord blood and cultured in the presence of macrophage colony-stimulating factor (M-CSF) and RANKL. Osteoclast differentiation was characterized by using tartrate-resistant acid phosphatase (TRAP) staining, giemsa staining, immunophenotyping, and reverse transcription-polymerase chain reaction (RT-PCR) assay for specific genes.

Results: Hematopoietic stem cells expressed RANK before and after differentiation into osteoclast. Compared to control group, flow cytometric results showed an increased expression of RANK after differentiation. Expression of CTR mRNA showed TRAP reaction was positive in some differentiated cells, including osteoclast cells.

Conclusion: Presence of RANKL and M-CSF in bone marrow could induce HSCs differentiation into osteoclast.

Keywords: Receptor Activator of Nuclear Factor-Kappa B, RANK Ligand, Hematopoietic Stem Cells, Osteoclasts, Calcitonin Receptor

Cell Journal (Yakhteh), Vol 18, No 3, Oct- Dec (Autumn) 2016, Pages: 322- 331

Citation: Kalantari N, Abroun S, Soleimani M, Kaviani S, Azad M, Eskandari F, Habibi H. Effect of the receptor activator of nuclear Factor κ B and RANK ligand on in vitro differentiation of cord blood CD133⁺ hematopoietic stem cells to osteoclasts. *Cell J.* 2016; 18(3): 322-331.

Introduction

Remodelling procedure of bone is composed of resorption and formation of this organ (1). Mesenchymal stem cells are responsible for osteoblast production during bone formation and osteoclasts are considered as bone-resorbing multinuclear cells originated from hematopoietic stem cells (HSCs) (2, 3). Physiologic condition establishes balance between osteoblast and

osteoclast activities. Misbalance in the production or activity of osteoclasts causes bone diseases such as multiple myeloma (MM) (1). MM is a hematologic disease (4-7), which is determined by the monoclonal expansion of malignant plasma cells in the bone marrow (BM) (8). Bone damages including bone lesion, spinal cord compaction and also bone fracture are hallmarks of myeloma bone disease (MBD), as po-